Environmental Health Perspectives
Vol. 21, pp. 79–84, 1977

Mutagenicity of Halogenated Alkanes and Their Derivatives

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The ability of a series of haloalkanes, haloethanols and haloacetaldehydes to induce mutations in *Salmonella typhrimurium* and preferentially to inhibit the growth of DNA polymerase-deficient *E. coli* (pol A^+/pol A^-) was investigated.

For the haloalkanes investigated, the order of reactivities towards the *E. coli* pol A^+/pol A^-, was: 1,1,2,2-tetrabromoethane > 1,1-dibromoethane > 1,1,2,2-tetrachlorethane > 1,2-dibromoethane = 1,5 dibromopentane > 1,2-dibromo-2-methylpropane > 1-bromo-2-chloroethane > 1,2-dichloroethane. In the standard *Salmonella* mutagenicity assay the order of these substances was 1,2-dibromoethane = 1,5-dibromopentane > 1,2-dibromo-2-methylpropane ≥ 1-bromo-2-chloroethane > 1,1,2,2-tetrachloroethane = 1,1-dibromoethane > 1,2-dichloroethane. 1,1,2,2-Tetrabromoethane was negative in the standard assay but strongly mutagenic when tested in suspension. It would appear that the discrepancy between the two procedures is due to the fact that bactericidal mutagens cannot be scored reliably in the standard *Salmonella* assay.

The order of reactivity of 2-haloethanols in *E. coli* pol. A^+/pol A^-, was 2-iodo > 2-bromo-> 2-chloroethanol. In the *Salmonella* assay the order was 2-bromo-> 2 iodo- >2-chloro-ethanol. 2-Fluoroethanol and ethanol were devoid of activity in both assays.

For the 2-haloacetaldehydes the reactivities in the *E. coli* system were 2-bromoethylacetate > 2-bromoacetaldehyde = acetaldehyde > 2-chloroacetaldehyde while in the *Salmonella* system the order was 2-bromoethylacetate > 2-chloroacetaldehyde. Acetaldehyde had minimal activity, while 2-bromoacetaldehyde was without activity but strongly bactericidal.

Because of their widespread use as lead scavengers in gasoline, fumigants, refrigerants, anesthetics, industrial solvents, and intermediates, the human population is widely exposed to halogenated olefins. For this reason our laboratory has for the last several years been involved in the study of the genetic and DNA-modifying properties of such agents (*1–4*). Moreover, because a number of halogenated alcohols are present as residues in fumigated food products and as contaminants in flame retardants, we have been interested in the genetic toxicology of this group of substances (*5–9*).

The findings reported herein are measures of DNA-modifying activity in living cells obtained using the *E. coli* DNA polymerase-deficient (*E. coli* pol A^+/pol A_1^-) system (*10*) and of mutagenicity as evidenced by genetic activity in the *Salmonella* mutagenicity assay (*11*).

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Results and Discussion

Haloalkanes

All of the haloalkanes examined inhibited the growth of the pol A_1^- strain preferentially (Table 1). The relative activities of these haloalkanes were determined by comparing the ratios of the areas of the zones of inhibition on pol A_1^- and pol A^+ strains. It was found that these values are independent of concentration provided that identical amounts are used for each strain. A ratio of 1.00 is indicative of a negative result (e.g., chloramphenicol), while values in excess of 1.00 indicate some preferential inhibition of the pol A_1^- strain. By these criteria it was found that tetrabromoethane and 1,1-dibromoethane were most active, while 1,2-dichloroethane was least potent. The other members of the group exhibited intermediate activities. It appeared that the bromoalkanes were more active than were their chloro analogs (1,2-dibromoethane vs. 1,2-dichloroethane, tetra-

Table 1. Effect of haloalkanes on the growth of DNA polymerase-deficient *E. Coli.*

Agent	Amount	Diameter of zones of inhibition, mm		Relative activity (area of pol A_1^-/area of pol A^+)[a]
		pol A^+	pol A_1^-	
1,2-Dibromoethane	10 μl	15	20	1.78
1,1-Dibromoethane	10 μl	14	23	2.70
1,2-Dichloroethane	10 μl	8	9	1.26
1-Bromo-2-chloroethane	10 μl	22	27	1.51
1,5-Dibromopentane	10 μl	12	16	1.78
1,2-Dibromo-2-methylpropane	10 μl	11	14	1.62
1,1,2,2-Tetrabromoethane	10 μl	19	35	3.39
1,1,2,2-Tetrachlorethane	10 μl	35	48	1.88
Methyl methanesulfonate	10 μl	45	54	1.44
Chloramphenicol	30 μg	28	28	1.00

[a]Relative activities were determined from the ratios of the areas of the zones of inhibition of the two strains. A value of 1.00 indicates lack of preferential inhibition of the pol A_4 strain. Data taken from Brem et al. (*1*).

bromoethane vs. tetrachloroethane). The mixed haloethane 1-bromo-2-chloroethane had an activity intermediary to those of 1,2-dibromoethane and 1,2-dichloroethane. When the bromine was on the same carbon, the biological activity (i.e., the ability preferentially to inhibit the pol A^-_1 strain) was enhanced (1,1-dibromoethane vs. 1,2-dibromoethane). When, however, the halogens were on different carbon atoms, the distance between them had no appreciable effect on the activity (1,2-dibromoethane vs. 1,5-dibromopentane).

The determination of relative mutagenicities of the haloalkanes requires the incorporation of known amounts of the agents into the agar overlay while it is still in the liquid (45°) phase. This proved impractical because of the volatile nature of some of these substances (*12*). To overcome this problem, the more qualitative assay was used. In this procedure the chemicals are deposited onto filter discs rather than directly on the surface of the agar.

When this procedure was used it was found that the number of revertant colonies per plate was a function of the amount of reagent added to the plate, rate of diffusion (Fig. 1), and size of the zone of growth inhibition. A series of typical plates is reproduced in Fig. 2. The data of Table 2, which are uncorrected for the size of the zones of growth inhibition, indicate that all of the haloalkanes tested, with the exception of 1,1,2,2-tetrabromoethane, are mutagenic for *S. typhimurium* TA 1530 and TA 1535. None of the substances induce mutations in TA 1538; i.e., those that are active induce mutations of the base-substitution type only. On the other hand, hydroxylaminoquinoline *N*-oxide, a known frameshift mutagen, induced revertants in strain TA 1538 (Table 2).

It is interesting and perhaps significant that the order of reactivities of these haloalkanes in the two microbial assay procedures is quite different. Thus in the *Salmonella* assay, 1,1,2,2-tetrabromoethane which was endowed with the greatest DNA-modifying activity was devoid of genetic activity, while 1,2-dibromoethane and 1,5-dibromopentane, which possess intermediate DNA-modifying activity, demonstrated the greatest mutagenic activity (Fig. 1). On the other hand, 1,1,2,2-tetrachloroethane, which displayed a DNA-modifying activity in the same range as the above two substances (Table 1), displayed only limited activity in *Salmonella typhimurium* (Fig. 1).

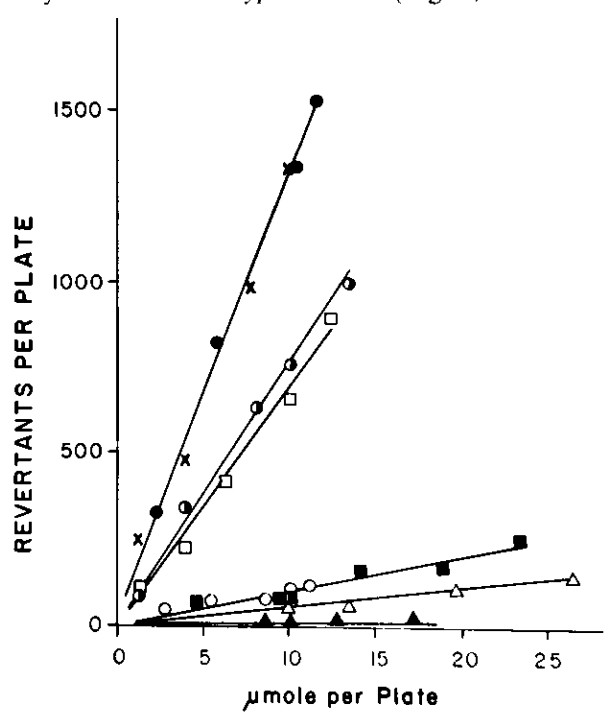

FIGURE 1. Effect of haloalkane concentration on mutagenicity for *S. typhimurium* TA 1530: (●) 1,2,-dibromoethane; (×) 1,5-dibromopentane; (◑) 1,2-dibromo-2-methylpropane; (□) 1-bromo-2-chloroethane; (■) 1,1,2,2-tetrachloroethane; (○) 1,1-dibromoethane; (△) 1,2-dichloroethane; (▲) 1,1,2,2-tetrabromoethane. Data of Brem et al. (*1*).

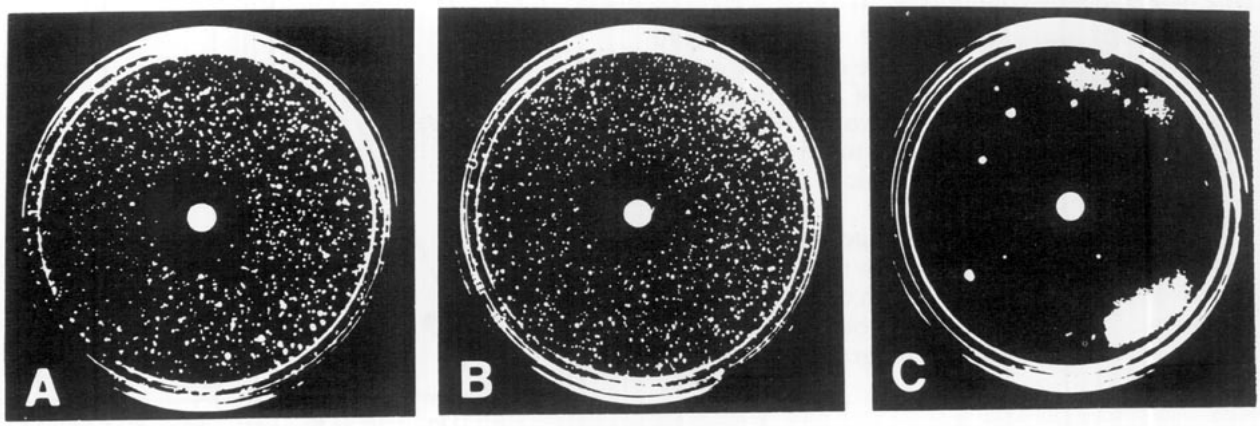

FIGURE 2. Mutagenicity of 1,2-dibromoethane for *S. typhimurium*. Minimal plates containing a trace of histidine received inoculations of (*A*) strain TA 1530, (*B*) TA 1535, and (*C*) TA 1538. A paper disc impregnated with 11.5 μmole 1,2-dibromoethane was deposited on the surface of each plate. The plates were incubated at 37°C for 54 hr and then examined for the appearance of histidine-independent colonies (mutants). Note the appearances of mutants in a zone surrounding the discs in the plates inoculated with *S typhimurium* TA 1530 and TA 1535 but not TA 1538. This indicates that 1,2-dibromoethane induces base substitutions but not frameshift mutations. Data of Brem et al. (*1*).

Table 2. Mutagenicity of haloalkanes for *Salmonella*.[a]

Agent	Amount	Revertants/plate	
		TA 1535	TA 1538
1,2-Dibromoethane	10 μmole	1438	18
1,1-Dibromoethane	10 μmole	63	19
1,2-Dichloroethane	10 μmole	54	19
1-Bromo-2-chloroethane	10 μmole	372	13
1,5-Dibromopentane	10 μmole	549	16
1,2-Dibromo-2-methylpropane	10 μmole	681	34
1,1,2,2-Tetrabromoethane	10 μmole	26	17
1,1,2,2-Tetrachloroethane	10 μmole	49	28
Methyl methanesulfonate	10 μl	552	37
Water	10 μl	26	19
Chloramphenicol	30 μg	31	14
4-Hydroxylaminoquinoline *N*-oxide	2.5 μg	49	99

[a]Data taken from Brem et al. (*1*).

The reason for most of these discrepancies between the two microbial assays is unknown (see, however, below for 1,1,2,2-tetrabromoethane). The discrepancies do, however, raise a number of questions concerning the usefulness of the assays as quantitative predictors of relative carcinogenicity (*13*).

The present data do, however, indicate that all of the haloalkanes tested give positive tests in one or the other of these assays or both. They further confirm the reported mutagenicity of 1,2-dibromoethane, 1,1-dibromoethane, and 1,2-dichloroethane (*14–19*). Because some of these substances are very widely used and have been shown to produce cancers in animals (*20, 21*), their continued unrestricted use seems unwise in view of the demonstrated relationships between ability to induce genetic effects in bacteria and cancers in animals (*22, 23*).

The finding that 1,1,2,2-tetrabromoethane displayed a potent ability preferentially to inhibit the *E. coli* pol A_1^- strain and yet was devoid of demonstrable mutagenic activity when tested in the standard *Salmonella* mutagenicity assay is reminiscent of the effect observed with other mutagens which possess antimicrobial activity (*12, 24–27*). In those instances, mutagenic activity could be readily demonstrated when the tester microorganisms were exposed for brief periods to the chemical in liquid culture. When cells are exposed to 1,1,2,2-tetrabromoethane and the frequency of mutation determined by plating on minimal media (to enumerate mutants) and on complete media (to enumerate survivors), the mutagenicity of this chemical

was readily demonstrated (Fig. 3).

It must be remembered that in the standard mutagenicity assay (*11*) results are expressed essentially as mutants per number of cells inoculated rather than as mutants per survivors. It seems that the ability of tetrabromoethane to devitalize cells extensively masks its mutagenicity in the plate assay system.

Because a number of test agents, some with known carcinogenicity, behave in the same manner (*12, 26–28*), it is suggested that, in order to decrease the number of false negatives, both procedures be used in tandem in screening programs.

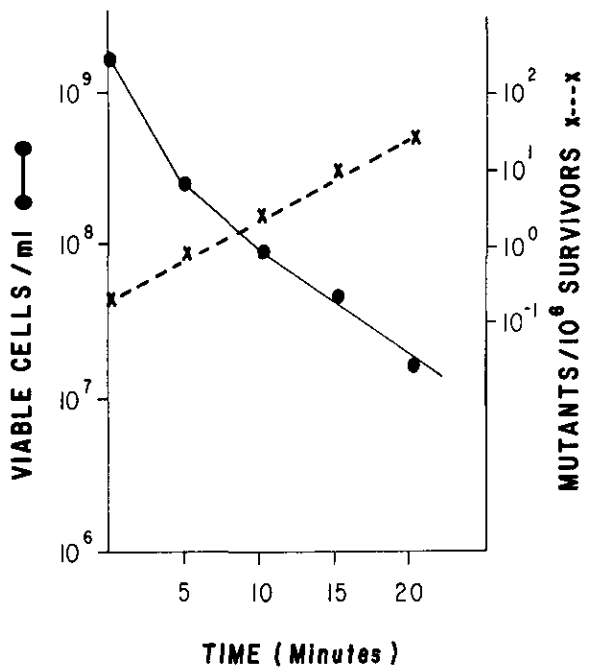

FIGURE 3. Mutagenicity of 1,1,2,2-tetrabromoethane for *Salmonella typhimurium* TA 1530. Bacteria were exposed to the test agent ($1.25 \times 10^{-4}M$). At intervals, bacteria were harvested, washed, and the number of mutants and viable cells determined. (This test agent was devoid of mutagenic activity when tested by the standard assay, see Table 2 and Fig. 1).

Haloethanols

2-Chloroethanol is a residue present in foodstuffs sterilized with ethylene oxide (*5, 6*). It has also been implicated as a metabolite of 1,2-dichloroethane (*19*), and of vinyl chloride (*29–33*) and as an intermediate in the chemical degradation of the therapeutically promising compounds 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrourea) (methyl-CCNU) (*34*).

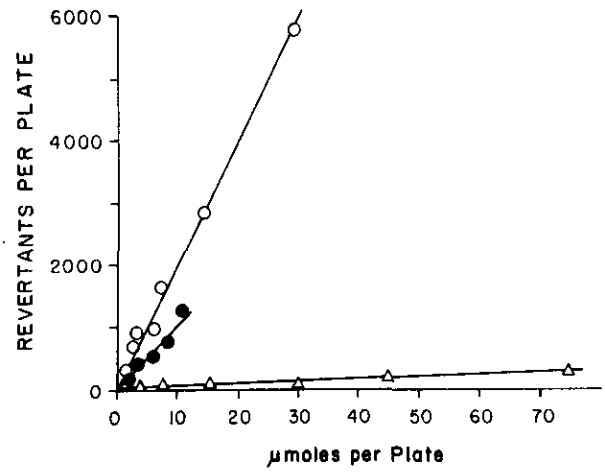

FIGURE 4. Effect of dose on mutagenic response of *S. typhimurium* TA 1530 to 2-haloethanols: (△) 2-chloroethanol; (●) 2-iodoethanol; (○) 2-bromoethanol (*6*).

The ability of 2-chloroethanol preferentially to inhibit *E. coli* pol A_1^- and to cause mutations of the base-substitution type was readily demonstrated (Table 3 and Fig. 4). This genetic effect has now been confirmed in a number of laboratories (*19, 29, 32*).

Investigation of the properties of the other 2-haloethanols revealed that ethanol and 2-fluoroethanol were without demonstrable DNA-modifying (Table 3) and mutagenic (unpublished results) potentials while the iodo and bromo derivatives possessed potent DNA-modifying and mutagenic properties (Table 3 and Fig. 4). On a molar basis, 2-iodoethanol displayed the most DNA-modifying activity, but 2-bromoethanol was most mutagenic. This discrepancy may well be a reflection of the fact that 2-iodoethanol is very bactericidal, and this may limit the expression of its mutagenicity (see Discussion, above).

2-Haloacetaldehydes

It has been suggested that 2-chloroethanol is metabolized to the corresponding aldehyde (*19*). There is, however, contraversy concerning the expression of the mutagenicity of this substance in *Salmonella* strains. Thus McCann et al. (*19*) reported that chloracetaldehyde is very mutagenic for strain TA 100 but not for strain TA 1535, while Malaveille et al. (*29*) and Rannug et al. (*32*) find that this chemical mutagenizes strains TA 1530 and TA 1535, respectively. In the present study it was found (Table 4) that 2-chloroacetaldehyde displayed some mutagenic activity towards strain TA 1535.

Table 3. Preferential inhibition of *E. coli* pol A_1^- by 2-haloethanols.[a]

Agent	Amount	Diameter of zones of inhibition, mm	
		pol A^+	pol A_1^-
Ethanol	10 μmole	0	0
2-Fluoroethanol	10 μmole	0	0
2-Chloroethanol	10 μmole	6.5	9.2
2-Bromoethanol	10 μmole	7.2	13.3
2-Iodoethanol	10 μmole	82.7	82.7
2-Iodoethanol	1 μmole	12.0	15.2
Propane sultone	250 μg	11.9	18.9
Chloramphenicol	30 μg	28	28

[a]Data from Rosenkranz et al. (6).

Table 4. DNA-modifying and mutagenic properties of 2-haloacetaldehydes.

Agent	Amount	Revertants/plate		Zone of inhibition, mm	
		TA 1535	TA 1538	pol A^+	pol A^-
2-Chloroethanol	10 μl	143	13	8	10
2-Bromoethanol	10 μl	2864	9	9	14
2-Chloroacetaldehyde[a]	10 μl	143	17	37	50
2-Bromoacetaldehyde[a]	10 μl	4	7	44	66
Acetaldehyde	10 μl	16	8	8	12
2-Bromoethylacelate	10 μl	210	10	9	14
Water	10 μl	4	7	0	0
Methyl methanesulfonate	10 μl			31	58
Ethyl methanesulfonate	10 μl	4000	6		
Chloramphenicol	30 μg			28	28

[a]Diethylacetal.

In view of our interest in the activity of other halogen congener, we investigated the properties of 2-bromoacetaldehyde. This agent exhibited no mutagenic activity at all for strain TA 1535 (Table 4), which again might be a reflection of its strong bactericidal action. On the other hand, chloroacetaldehyde as well as bromoacetaldehyde preferentially inhibited the pol A_1^- strain (Table 4). This was a property also exhibited—although to a lesser extent—by the parent acetaldehyde. [This last observation is not too surprising in view of the fact that formaldehyde also preferentially inhibits the growth of the pol A_1^- strain (*35*)]. Unlike 2-bromoacetaldehyde, 2-bromoethyl acetate exhibited mutagenic activity for strain TA 1535 (Table 4).

It would be most interesting to pursue further the chemical basis of the mutagenic action of these haloethanols and haloacetaldehydes. Thus it is unlikely that the mutagenic activity of the haloethanols (e.g., bromoethanol) derives only from their conversion to the corresponding aldehydes as these acetaldehyde are frequently nonmutagenic. It may well be that in animals the enzymic activity is present to accomplish these biotransformations. However, in bacteria it is quite possible that with haloethanols we see the result of alkylation reactions, while with the corresponding aldehydes we record the formation of adducts involving the 6-position of the purine and pyrimidine rings (*36*, *37*). Certainly these possibilities warrant further exploration.

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